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METHOD FOR DETECTING PREMATURE FLOCCULATION
[SOGYOSEI HANTEI HO]

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Specification

Method for detecting premature flocculation

Technical Field

The present invention relates to a method for detecting the premature flocculation of a raw material cereal, which is used for brewing beer, whisky et cetera, and more specifically, the present invention relates to a method for detecting the presence of the premature flocculating factor in the raw material cereal.

Background Art

Alcoholic beverages which are produced by fermenting a raw material malt of cereals, typified by barley and wheat, by using yeasts include beer (including beverages which are classified into "happoshu" according to the liquor tax law due to their lower malt content than beer) and whiskies.

During the production of such alcoholic beverages using malt as a raw material, in some cases, a phenomenon that is referred to as "premature flocculation" is observed in the fermentation step. This phenomenon refers to a case in which yeasts flocculate and precipitate despite the fact that utilizable sugar in the yeast still remains in the wort in the

fermentation step, particularly in the late stage of the fermentation. When yeasts flocculate and precipitate, the fermentation process stops. If this phenomenon occurs, fermentation will be insufficient; therefore, the resulting products may become off-specification alcoholic beverages and, when a cereal having premature flocculating properties is brewed to produce beer, et cetera, it is known that a significant loss is caused.

In order to solve the problem with this premature flocculation phenomenon in the production of alcoholic beverages using malt as a raw material, many studies have been previously undertaken; as a result, although it has been revealed that the premature flocculation phenomenon is caused by high-molecular-weight acidic polysaccharides included in malt, derived from the raw material cereal, it has not yet been identified whether the factors inducing the premature flocculation phenomenon (which are referred to as "premature flocculating factors", hereinafter) are present in the raw material cereal

or are generated during the malting process. Further, studies concerning a method of preventing the premature flocculation phenomenon by eliminating the premature flocculating factors are currently not so well served.

In the case of using barley as a raw material, it has been conventional practice to detect the presence of the premature flocculating factors in the barley so that only the barley malt which does not induce the premature flocculation is used. The conventional method for detecting the presence of the premature flocculating factors in the barley is conducted by malting the barley on a small scale, preparing the wort from the resulting malt, fermenting the wort by using enzymes, and detecting the presence of the premature flocculation of the barley according to the progress status of the fermentation.

The above described conventional method allows the presence of the premature flocculating factors in the barley to be detected by making the malt on a scale small. Although the reliability of the results is satisfactory, about seven days are required for malting and the preparation of the wort and about eight days are required for detecting the presence of the premature flocculation of the barley according to the progress status of the fermentation; therefore, a total of half a month is required for detecting the presence of the premature flocculation of the barley according to the progress status of the fermentation.

In addition, since resting, the water sensitivity etc of barley must be considered for malting, barley cannot be subjected to malting immediately after harvest; therefore, the

above described conventional method is not applied to barley immediately after harvest, but the detection of the presence of the premature flocculation of the barley is only possible after about two months have passed, which is when malting can be conducted. Hence, at least two months is required to detect the presence of the premature flocculation of the harvested barley; in particular, in the case of barley immediately after harvest, since it is impossible to detect the possibility of premature flocculation in the premature stage, when barley having premature flocculating properties is purchased, a significant loss is caused.

Disclosure of the Invention

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The object of the present invention is to provide a method which is quite simple as compared with the above described conventional method and allows the detection of premature flocculation properties in the raw material cereal in a short period of time, in other words, the detection of the presence of the premature flocculating factor in the raw material cereal.

During the study of the premature flocculation phenomenon, the present inventors discovered that the premature flocculating factor inducing the premature flocculation phenomenon was not generated in the malting step but was originally present in the

raw material cereal. Specifically, the present inventors discovered that, when an enzyme treatment was conducted by adding a specific enzyme to the raw material cereal, the premature flocculating factor was extracted without being decomposed and, further, the detection of the possibility of the premature flocculation was possible by conducting the fermentation test using the resulting enzymatically-treated material. The present invention has thus been completed.

More specifically, the present invention relates to a method for detecting the premature flocculation of the raw material cereal in which the premature flocculation of a raw material cereal, such as barley, is detected by a fermentation test using a yeast, characterized in that the raw material cereal is subjected to an enzyme treatment by using enzymes, preferably enzymes containing at least a combination of enzymes chosen from among α-amylase, β-amylase, β-glucanase and protease, and the resulting enzymatically-treated material is used as a part of whole of the raw material for the fermentation test.

Further, the present invention relates to a method for detecting the premature flocculation of the raw material cereal in which the premature flocculation of the raw material cereal, such as barley, is detected by a fermentation test using a yeast, characterized in that the raw material cereal is subjected to an enzyme treatment by using enzymes, preferably enzymes containing

at least a combination of enzymes chosen from among α-amylase, β-amylase, β-glucanase and protease, and the macromolecule fraction is separated from the resulting enzymatically-treated material so as to be used as a part of the raw material for the fermentation test.

Furthermore, the present invention relates to a method for detecting the premature flocculation of the raw material cereal in which the premature flocculation of the raw material cereal, such as barley, is detected by a fermentation test using a yeast, characterized in that the raw material cereal is subjected to an enzyme treatment by using enzymes, preferably enzymes containing at least a combination of enzymes chosen from among α-amylase, β-amylase, β-glucanase and protease,

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and either the resulting enzymatically-treated material or the macromolecule fraction which is separated from the resulting enzymatically-treated material is added to a synthetic wort to obtain a raw material for the fermentation test so that the turbidity of the raw material for the fermentation after 48 hours can be determined, thereby determining the presence of the premature flocculating factor in the raw material cereal.

In the present invention, the raw material cereals, such as barley and wheat, are not restricted to cereals which have been rested for a certain period of time after harvest so as to be

malted but cereals immediately after harvest can also be used. The required amount of the raw material cereals for detecting the possibility of premature flocculation is about 20 g, but it is preferred that the amount is about 50 g in order to improve the accuracy of the test.

It is preferred that the raw material cereals are crushed so that the enzyme treatment can be efficiently conducted, in other words, so that 90 wt % of more of the raw material can pass through a sieve having a hole diameter of 0.547 mm. Next, the crushed raw material is suspended in water, preferably warm water having a temperature of from 40 to 60 degrees Celsius, and is then mixed with an enzyme (enzyme preparation) so as to be subjected to an enzyme treatment.

In the present invention, the enzymes to be added are not restricted as long as they allow the premature flocculation factor to be extracted from the raw material cereals without being decomposed; however, enzymes containing at least a combination of enzymes chosen from among α-amylase, β-amylase, β-glucanase and protease are preferred.

The enzymes to be added are not restricted to high-purity enzymes as long as they have an enzymatic activity; for example, enzyme-containing processed bacteria can be used and, further, a mixture of enzymes having different activities can also be used, provided that they do not decompose the premature flocculating

factor and prevent the crushed raw material cereals from being gelatinized in water.

As for enzymes that can be used, examples of enzymes containing α-amylase, protease and β-glucanase include

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"Ceremix 6XMG" (produced by Novozymes); examples of enzymes containing β-glucanase include those derived from Aspergillus niger (trade name: "Finizym" produced by Novozymes); and examples of enzyme containing β-amylase include those derived from barley (β-amylase produced by Sigma Chemical Co.)

Conditions for the enzyme treatment, such as the amount of enzymes added, the reaction temperature and the reaction time, may vary depending on the characteristics of the enzymes used, but it is preferred that the reaction is conducted under conditions such that the enzymatic activities can be sufficiently produced. For example, when the above-enumerated enzyme is used, the reaction can be sufficiently conducted at a temperature in a range of from 40 to 60 degrees Celsius for about 2 to 3 hours or more. Obviously, the reaction temperature can be stepwisely varied as in the case with the conventional saccharification process.

The enzymatically-treated material obtained by the enzyme treatment is first filtered through a filter paper sheet or the like, is heated by a boiling method or the like so as to

deactivate the enzymatic activity, and is filtered again so that the thermal coagulant produced due to the previous heating is removed. When the raw material cereals have premature flocculation properties, since the premature flocculating factor is included in the macromolecule fraction in the filtrate (which is referred to as "pseudo wort", hereinafter), the pseudo wort, without being additionally processed or being mixed with other appropriate fermented materials, is subjected to a common fermentation test using an yeast, thereby allowing the detection of the presence of the flocculating factor in the raw material cereals.

Further, when only the macromolecule fraction which has been separated from the pseudo wort is added, as a part of the raw material, to another ferment raw material for the fermentation test, the presence of the premature flocculating factor can be more precisely detected. Specifically, the macromolecule fraction can be separated, for example, by the following method: the pseudo wort is mixed with ethanol, the amount of which is twice the amount of the pseudo wort, and the resulting mixture is then stirred for about 5 minutes so that the macromolecule fraction is obtained as precipitate. In addition to this precipitation method, any separation methods, such as dialysis and ultrafiltration, which allow the

macromolecule fraction to be separated from the pseudo wort can also be used.

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When the raw material cereals have premature flocculation properties, the premature flocculating factor is included in this macromolecule fraction.

Although commonly used wort can be used as a medium where the macromolecule fraction for the fermentation test, it is preferred that a synthetic wort (e.g., Weinfurtner, F., et. al. Brauwissenschaft, 14, 109 (1961)) is used which is prepared by using constituents other than malt (e.g., sugar, amino acids and inorganic salts) in order to obtain the result precisely with good repeatability. The preparation method for a synthetic wort, which is obtained by partially modifying a synthetic wort, such as Weinfurtner, is described below.

(Preparation of synthetic wort)

Solution A, solution B, solution C, and solution D, each having the following formulation, are each independently prepared in advance. As for solution C and solution D, stock solutions are prepared, respectively, and stored in a refrigerator so that they can be dispensed in a clean bench.

Next, 700 mL of solution A, 50 mL of solution B, 5 mL of solution C and 10 μL of solution D are mixed, and purified water

is then added to make the total volume 800 mL and to adjust the pH to be 5.7.

Solution A:

D (-) - fructose	2 g
D (+) - glucose	8 g
Sucrose	4 g
Maltose - hydrate	64 g
Dextrin	27 g
Casamino acid	3.5 g
Peptone	4 g
$CaCl_2$ (anhydride)	1 g

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KCl	1 g

The above-enumerated constituents are dissolved in purified water to make the total volume 700 mL and the resulting solution is sterilized in the autoclave.

Solution B:

$MgSO_4 \cdot 7H_2O$	1 g

The above-enumerated constituent is dissolved in purified water to make the total volume 50 mL and is then sterilized in the autoclave.

Solution C:

Inositol	500 mg
(+) - calcium pantothenate	500 mg

Nicotinic acid	50 mg
Thiamine hydrochloride	50 mg
Pyridoxal hydrochloride	50 mg
(+) - biotin	50 mg
Uracil	25 mg
Guanine	25 mg

The above enumerated constituents are dissolved in purified water to make the total volume 250 mL and are then filter-sterilized.

Solution D:

H_3BO_3	100 mg
$ZnSO_4.7H_2O$	100 mg
$MnCl_2.4H_2O$	100 mg
$FeCl_3$	50 mg
$CuSO_4.5H_2O$	10 mg
KI	10 mg

The above enumerated constituents are dissolved in purified water to make the total volume 1,000 mL and are then sterilized in the autoclave.

A conventional method (K. Morimoto, et. al. Rept. Res. Lab. Kirin Brewery Co., Ltd., 18, 63 (1975)) can be used

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as the detection method after the fermentation test. The conventional method can be conducted, for example, by preparing

a fermentation test raw material (medium) from the pseudo wort or by preparing the fermentation test raw material (medium) from a mixture of the wort obtained from raw material without premature flocculation properties and the pseudo wort or the macromolecule fraction, allowing the resulting medium to be fermented at a temperature of about 8 degrees Celsius for about 8 days using yeasts, and comprehensively determine the growth rate of the yeasts according to the turbidity or sugar content in the raw material (medium).

In particular, when the macromolecule fraction is used for the fermentation test, precise detection can be conducted by measuring the turbidity after the medium has been fermented at a temperature of 20 degrees Celsius for 48 hours. In this case, the optical density at a wavelength of 800 μm (in other words, OD 800) is measured by using a turbidity meter so as to be compared with the value DPF (degree of premature flocculation) 48, which is obtained by substracting the OD 800 of the testing area from the OD 800 of the control area. It is preferred that normal cereals or premature flocculated cereals are simultaneously tested as a control so that the resulting turbidity is also compared.

Best Modes of Implementing the Invention

The following embodiment illustrates the present invention, but does not restrict it in any way.

Embodiment

0.5 g of each commercially available enzyme containing β-glucanase ("Finizym" produced by Novozymes) and commercially available enzyme containing protease, α-amylase and β-glucanase ("Ceremix 6X MG" produced by Novozymes) and 500 units of β-amylase ("β-amylase" produced by Sigma Chemical Co.) were added to 300 mL of warm water having a temperature of 55 degrees Celsius,

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the resulting mixture was mixed with 50 g of barley, which had been finely crushed using a disk mill, was then thoroughly stirred to obtain a uniform solution, and was left at rest at a temperature of 55 degrees Celsius for 3 hours in order to complete the enzyme treatment, thereby obtaining an enzymatically-treated material.

The resulting enzymatically-treated material was thoroughly stirred, was then filtered through a filter paper sheet (Toyo Filter Paper No. 2), and 180 mL of the filtrate was precisely dispensed. 180 mL of the resulting dispensed filtrate was heated to boil until the volume became a half or less, and the volume of the filtrate was then made to make 100 mL and was then

filtered out by the same method as above, thereby obtaining the pseudo wort.

200 mL of ethanol was slowly added to the pseudo wort while it was being stirred. After being stirred for 5 minutes, the resulting mixture was centrifuged, the supernatant fluid was removed, the resulting precipitate was mixed with 10 mL of boiling water so as to be dissolved therein, the volume of the resulting solution was made to make 25 mL, the centrifugation was conducted again, and the supernatant liquid (which is referred to as "the extract of the barley macromolecule fraction extract", hereinafter) was subjected to the fermentation test.

20 mL of the resulting extract of the barley macromolecule fraction was added to 80 mL of a synthetic wort, which had been prepared by the above described method, and the pH of the resulting solution was adjusted to be 5.7, thereby obtaining a testing area. Meanwhile, the same method as above was conducted, except that purified water was used instead of the extract of the barley macromolecule fraction, and the pH of the resulting solution was adjusted to be 5.7, thereby obtaining a control area. The resulting areas to which 0.35 g of brewery yeast had been added were placed in fermentation tubes (volume 100 mL) having a diameter of 27 mL so as to be fermented at a temperature of 20 degrees Celsius. After 48 hours, 2 mL of each liquid was dispensed from 5 cm away from the liquid level in

order to measure the OD 800, respectively. The DPF 48 was thereby obtained by substracting the value of the testing area from the value of the control area.

The correlation with the actual premature flocculation properties was investigated by using in-situ cereals, wherein 7 types of barley of which the presence of the premature flocculation had been detected were used as samples for the above described test. The results are shown in Table 1. As is clear from the results shown in Table 1, a high correlation was found to be observed between the DPF 48 value and the actual premature flocculation properties; it was confirmed that the barley having the premature flocculation properties had a greater DPF 48 value. In addition, the repeatability of the results was found to be quite high.

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[Table 1]

Sample barley No.	DPF 48 according to the present invention	In-situ tests by conventional method
1	0.29	No premature flocculation found
2	0.29	No premature flocculation found
3	0.36	No premature flocculation found
4	0.36	No premature flocculation found
5	0.66	Premature flocculation found
6	0.70	Premature flocculation found
7	0.75	Premature flocculation found

Potential Industrial Applications

In accordance with the present invention, the premature flocculation properties of the raw material cereals can be

precisely detected within about 5 days with good repeatability. Further, the present invention enables the detection of the premature flocculation of the raw material cereals immediately after harvest or using only a small amount of the raw material cereal.

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Scope of Patent Claims

1. A method for detecting premature flocculation of a raw material cereal, in which the premature flocculation of the raw material cereal is detected by a fermentation test using a yeast, characterized in that the raw material cereal is mixed with an enzyme so as to be enzymatically treated, and the resulting enzymatically-treated material is used as a part or whole of the raw material for the fermentation test.

2. A method for detecting premature flocculation of a raw material cereal, in which the premature flocculation of the raw material cereal is detected by a fermentation test using a yeast, characterized in that the raw material cereal is mixed with an enzyme so as to be enzymatically treated, and the macromolecule fraction is separated from the resulting enzymatically-treated material so as to be used as a part of the raw material for the fermentation test.

3. A method for detecting premature flocculation of a raw material cereal, in which the premature flocculation of the raw material cereal is detected by a fermentation test using a yeast, characterized in that the raw material cereal is mixed with an enzyme so as to be enzymatically treated and either the resulting enzymatically-treated material or the macromolecule fraction which is separated from the resulting enzymatically-treated material is added to a synthetic wort to obtain a raw material for the fermentation test so that the turbidity of the raw material for the fermentation after 48 hours can be determined, thereby determining the presence of the premature flocculating factor in the raw material cereal.

4. The method for detecting premature flocculation of a raw material cereal according to any one of Claims 1 through 3, characterized in that the enzyme treatment is conducted by using enzymes containing at least a combination of enzymes chosen from among α-amylase, β-amylase, β-glucanase, and protease.

5. The method for detecting premature flocculation of a raw material cereal according to any one of Claims 1 through 4, characterized in that the raw material cereal is barley.